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Paired measurements of total and unconjugated estriol in maternal plasma during the 2nd and 3rd trimester of pregnancy – their relationship to intrauterine growth retardation

Giovanni Bacigalupo, Erich Z. Saling, Jörg Gesche

Unit of Perinatal Medicine – The Free University of Berlin

Growth retarded fetuses and neonates have higher morbidity and mortality rates than normally developed infants of comparable age [4, 5, 16, 19, 20, 24]. During the immediate neonatal period, small-for-date infants run an increased risk of infection, are particularly prone to hypoglycemia and have difficulties in maintaining normal body temperature. Subsequently some of these infants will not attain normal body size, and a considerable number will suffer from neurologic impairment [8, 10, 23].

Early detection of intrauterine growth retardation (IUGR) and careful monitoring of the affected fetuses, both by biophysical and biochemical means, are of substantial interest to the obstetrician. Once fetal growth retardation is recognized, the obstetrician will attempt to normalize fetal growth during the further course of the pregnancy by elimination of maternal causes contributing to the gestational disorder (nicotine, drug abuse, maternal malnutrition, etc.) or by intensive therapy of accompanying maternal complications (toxemia, gestational diabetes, etc.) In some cases, early delivery of a viable fetus will be accomplished before irreversible damage or death has occurred.

During the 2nd and 3rd trimester of pregnancy, the major proportion of estriol circulating in maternal blood and being excreted through maternal urine and feces is synthesized in the feto-placental unit [6, 7]. Normal maternal plasma estriol levels indicate fetal well-being, whereas abnormal estriol profiles are mostly associated with abnormal fetal development or fetal distress [14].

The study was designed to examine the possibilities of predicting fetal growth retardation as early as during the 2nd trimester of pregnancy by means of total and unconjugated estriol assay in maternal plasma and to compare the usefulness of these two feto-placental function tests in the antepartum identification of growth retardation.

1 Patients and materials

In 583 pregnancies at 15 to 42 gestational weeks, 913 plasma samples of peripheral maternal venous blood were assayed for their concentrations of total estriol and unconjugated estriol.

Among the pregnancies examined there were 463 mothers who were delivered of a healthy singleton infant of normal birthweight. These women contributed 707 plasma samples to this study. Normal birthweight was defined as being between the 10th and 90th percentiles for gestational age according to the birthweight curves of NICKL [25], which describe the relative frequency of birthweights by week of gestation for the German population in the 1960s. Gestational age was determined by the clinical criteria proposed by FARR [9] and PETRUSSA [26] which are based upon estimations of neonatal maturity.

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120 pregnant women examined gave birth to singleton infants whose birthweight fell below the 10th percentile for gestational age and who were therefore classified as suffering from intrauterine growth retardation. From the mothers with an IUGR pregnancy, 206 plasma samples were assayed for estriol. Comparative clinical data from both the normal weight and the IUGR neonates as well as some peculiarities in these pregnancies and deliveries are given in Tabs. I and II.

Tab. I. Clinical data on the cases examined. *) Arithmetical mean and standard deviation.

	Neonates with normal weight	Neonates with intrauterine growth retardation
Body weight	4115*) ± 411 g	2440 ± 462 g
Placental weight	620 ± 114 g	492 ± 109 g
BALING score (points)	10,1 ± 1,1	8,9 ± 2,0
Vaginal delivery	89,8% (416)	89,2% (107)
Cesarean section	10,2% (47)	10,8% (13)
Age of mothers	28,5 ± 6,1 yrs.	27,1 ± 5,9 yrs.
Number of cases examined	463	120

For estriol assays 2 ml of maternal peripheral venous blood were routinely withdrawn in heparinized plastic syringes and gently shaken. After centrifugation the plasma layers were removed and stored at − 20 °C until their processing, which took place within one week after withdrawal.
In each plasma assay, paired determinations of total and unconjugated estriol were made applying the same radioimmunologic procedure. Analyses of both estriol levels (total and unconjugated) in the same sample were carried out in one and the same RIA run. All of the estriol determinations were performed in duplicate; thus the study was based on 1652 single measurements.

Tab. II. Gestational disorders (A) and neonatal disturbances immediately after delivery (B) in the cases examined. * In parentheses real numbers. Respiratory acidosis = pH act. ≤ 7,19 and CO_2 overload in arterial cord blood. Metabolic acidosis = pH qu40 ≤ 7,19 in arterial cord blood. Neonatal depression = ≤ 6 points of the BALING score [27]; the scoring criteria of BALING are similar to those of APGAR.

	Neonates with normal weight	Neonates with intrauterine growth retardation
A. Gestational diabetes	4,1% (19)*	10,0% (12)
Juvenile diabetes mellitus	0 (0)	0,8% (1)
Toxemia	3,2% (15)	10,9% (13)
B. Respiratory acidosis	8,2% (38)	17,5% (21)
Metabolic acidosis	4,3% (20)	8,3% (10)
Neonatal depression	1,3% (6)	3,3% (4)

1.1 Hormone assays

For total estriol assay, plasma samples were subjected to enzyme hydrolysis. For that purpose a preparation of beta-glucuronidase/arylsulphatase from Helix pomatia (5,2 and 2,6 U/ml) was used (Boehringer/Mannheim, Inc.). 0,02 ml plasma, 0,02 ml enzyme solution and 0,2 ml 0,1 N acetate buffer pH 4,6 were mixed in stoppered glass tubes and incubated at 37 °C for 90 minutes. Complete hydrolysis of the estriol conjugates was achieved, as was shown in repeated tests with pure estriol conjugates in aqueous solution and in plasma samples with known estriol content. Total estriol was then extracted by adding to the hydrolyzed pre-chilled samples 3 ml of highly purified diethylether ("Uvasol", E. Merck, Inc., Darmstadt) from freshly opened bottles, by Vortex mixing for 3 minutes and centrifugation at 2000 g and a temperature of 2 °C for 10 minutes. To each of 2 RIA tubes, 0,1 ml of the ether layer containing the extracted total estriol was transferred, evaporated in a vacuum at 50 °C, and the remainder was thoroughly dried.
Unconjugated estriol was extracted from 0,02 ml plasma with 3 ml ether. Subsequently 1 ml of the supernatant ether phase was given to each of 2 RIA tubes, evaporated and thoroughly dried, as

described. The extraction yield amounted to 98.1 ± 1.2% as measured by the recovery of known quantities of tritium-labelled estriol.

Estriol quantitation was carried out by means of a radioimmunoassay which followed in essence the procedure described by TULCHINSKY and ABRAHAM [29]. The estriol extracted was incubated with estriol antiserum and labelled estriol in 0.04 M phosphate buffer pH 7.4 with 0.1% bovine serum albumin added at 0° to 4 °C for 120 minutes. Unbound estriol was then separated from antiserum bound estriol by the addition of a charcoal/dextran suspension (1 mg charcoal per tube). After centrifugation the radioactivity of the supernatant was measured in a liquid scintillation counter. The estriol concentrations of the samples assayed were calculated using a special computer program.

The estriol antiserum used was raised in rabbits through repeated injections of a conjugate of estriol-6-(O-carboxymethyl)oxim with BSA as immunogen (SORIN BIOMEDICA, Saluggia, Italy). This antiserum had a cross-reaction of 0.01% with estradiol and 0.001% with estrone at 50% displacement. As tracer 2,4,6,7-^{3}H-(N) estriol was used up to 0.5 μCi (18.5 KBq) for 100 samples.

In the range of 10 and 100 pg estriol per RIA tube, the intra-assay and inter-assay variation coefficients were 5.1% and 6.9%. Using the RIA system, the lowest detectable amount of estriol was 6 ± 2 pg. The recovery of known estriol amounts added to plasma samples was at 96% nearly quantitative.

2 Results and comments

2.1 Total estriol in pregnancies with normal outcome

Tab. III and Fig. 1 show the total estriol concentrations (conjugated plus unconjugated steroid) in maternal plasma related to gestational age for the

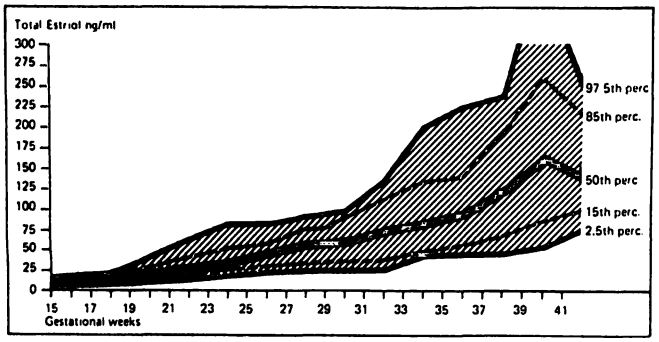

Fig. 1. Percentile curves of total estriol in maternal plasma related to gestational age in pregnancies with healthy, normal weight infants.

Tab. III. Means, medians, 95% and 70% confidence limits, and ranges of total estriol determined in 707 maternal plasma samples from 463 pregnancies with normal weight, healthy neonates. 95% confidence limits = between 2.5th and 97.5th percentiles, 70% confidence limits = between 15th and 85th percentiles. Conversion to SI units: 1 ngE3/ml = 3.47 nmolE3/l.

Gestational weeks	Number of plasmas assayed	Total estriol (ng/ml) Mean	Median	95% C.L.	70% C.L.	Min.-Max.
15–16	28	7.94	6.81	3– 17	5– 11	2– 25
17–18	51	11.67	10.93	5– 19	8– 16	3– 30
19–20	60	18.30	16.74	8– 38	12– 25	6– 40
21–22	41	27.07	24.62	11– 59	15– 38	8– 59
23–24	61	34.14	28.49	17– 79	21– 49	13– 82
25–26	52	41.41	41.10	19– 80	27– 54	17– 90
27–28	63	52.53	53.31	21– 92	30– 74	16– 99
29–30	30	57.75	55.40	21– 94	34– 85	17– 96
31–32	28	72.24	71.24	22–133	40–106	17–136
33–34	57	89.64	81.03	39–201	45–131	37–237
35–36	75	99.20	93.98	39–225	51–136	30–282
37–38	95	127.06	118.50	43–259	64–187	36–361
39–40	51	163.52	160.70	53–405	84–260	48–432
41–42	15	144.15	139.61	73–234	96–210	64–241

pregnancies with normal weight and healthy outcome. Taken as a whole, the values were similar to the reference values recently reported by FRESINSKY, CASTANIER, GRENIER and SCHOLLER [11]. Due to the fact that there were asymmetrical distributions of the hormone concentrations, the estriol figures were represented as percentiles. As values of comparison to the medians, the appropriate arithemetical means were given in the estriol tabulation. The median total estriol concentrations were in general slightly lower than their means, indicating a denser aggregation of the estriol values below the means than above them. Commencing with the gestational weeks 35 to 36, the well-known steep rise in total estriol concentrations could be noticed. This was associated with a wide scatter of single values up to and above 400 ng/ml.

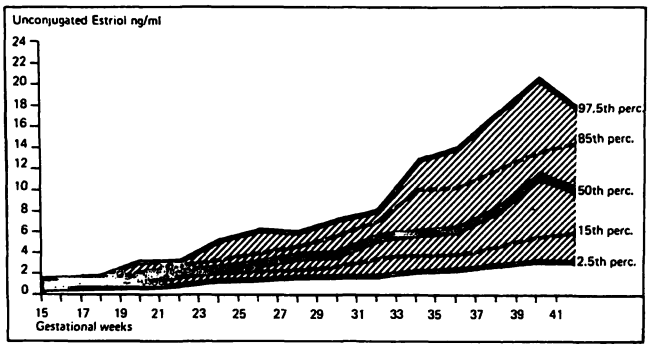

Fig. 2. Percentile curves of unconjugated estriol in maternal plasma related to gestational age in pregnancies with healthy, normal weight infants.

The 15th percentiles for total plasma estriol which were used as thresholds in the characterization of abnormally low steroid levels and as upper screening limits of a fetal danger zone, showed a marked increase from 5 to 21 ng/ml early in the 2nd trimester. They then lay between 25 and 40 ng/ml in the gestational weeks 25 to 32, and rose to values of approximately 45 to 65 ng/ml between weeks 33 and 38. Later on, the 15th percentiles of total plasma estriol varied between 85 and 95 ng/ml.

2.2 Unconjugated estriol in pregnancies with normal outcome

Tab. IV and Fig. 2 show the unconjugated estriol concentrations in maternal plasma related to gestational age. By comparison of Fig. 1 and 2 one may easily recognize that there was a close parallel in the shape of the curve ascents for unconjugated and total plasma estriol during the second and third trimester, with the average proportion of unconjugated estriol varying between 6 and 8% of total estriol. The above-mentioned steep rise of total plasma estriol at gestational weeks 35 to 36 with an associated very wide scatter of some individual values also occurred in the unconjugated estriol fraction.

The 15th percentiles for unconjugated estriol in maternal plasma rose steeply from 0.3 to 1.7 ng/ml between gestational weeks 15 and 24. During the

Tab. IV. Means, medians, 95% and 70% confidence limits, and ranges of unconjugated estriol in maternal plasma from the pregnancies with normal weight, healthy neonates.

Gestational weeks	Number of plasmas assayed	Unconjugated estriol (ng/ml)				
		Mean	Median	95% C.L.	70% C.L.	Min.-Max.
15–16	28	0.64	0.59	0.2– 1.5	0.3– 1.0	0.13– 1.64
17–18	51	0.88	0.80	0.3– 1.6	0.6– 1.2	0.26– 2.05
19–20	60	1.45	1.36	0.5– 3.0	0.8– 2.0	0.46– 3.90
21–22	41	1.94	2.00	0.8– 3.0	1.3– 2.6	0.65– 3.38
23–24	61	2.40	2.13	1.3– 5.2	1.7– 3.1	0.90– 5.79
25–26	52	3.04	2.64	1.4– 6.1	2.1– 3.8	1.32– 9.05
27–28	63	3.51	3.54	1.6– 5.8	2.4– 4.6	1.44– 6.52
29–30	30	3.97	3.73	1.6– 7.2	2.4– 5.6	1.09– 9.37
31–32	28	5.05	5.25	1.5– 7.9	3.4– 6.8	1.01– 8.40
33–34	57	6.57	6.00	2.0–12.9	3.8–10.5	1.60–14.10
35–36	75	7.00	6.02	2.4–13.8	3.6–10.7	2.01–23.70
37–38	95	8.34	7.87	2.7–17.0	4.5–11.9	2.33–20.40
39–40	51	9.48	11.07	3.0–20.5	5.3–13.3	2.65–25.20
41–42	15	9.17	9.87	3.0–17.8	5.6–14.3	3.28–18.90

weeks 25 to 32 they were 2.1 to 3.4 ng/ml; in the weeks 33 to 38, 3.8 to 4.5 ng/ml; and 5.3 to 5.6 ng/ml during the last four gestational weeks.

2.3 Total estriol in pregnancies with intrauterine growth retardation

Tab. V shows the total estriol concentrations in maternal plasma related to gestational age for the pregnancies with small-for-date babies. From comparison of these values with those in pregnancies with normal outcome, it becomes clear that IUGR was associated with low concentrations of maternal plasma estriol, both in the 2nd and in the 3rd trimester. The total estriol medians in the IUGR group lay, with the exception of two values during early 2nd trimester, below those in the reference group. The values strongly diverged especially during the last 6 to 8 weeks of gestation. In this period, the total estriol median curve of the IUGR group lacked the characteristic accelerated increase to be found in the group of pregnancies with normal outcome (Fig. 3).

The absolute and relative distribution of the individual total estriol concentrations measured in the IUGR group within the above-described estriol percentiles system from the pregnancies with normal outcome is represented in Tab. VII. In the IUGR group (gestational weeks 15 to 42) 36.40% (14.56% plus 21.84%) of total estriol values moved below the 15th total estriol percentile of the reference group with normal outcome. Remarkably, during the 3rd trimester (gestational weeks 29 to 42) the relative frequency of total estriol values from the IUGR group below the 15th reference estriol percentile was 41.27% (16.67% plus 24.60%); whereas, during the 2nd trimester (gestational weeks 15 to 28), it was only 28.75% (11.25% plus 17.50%) (Tab. VII).

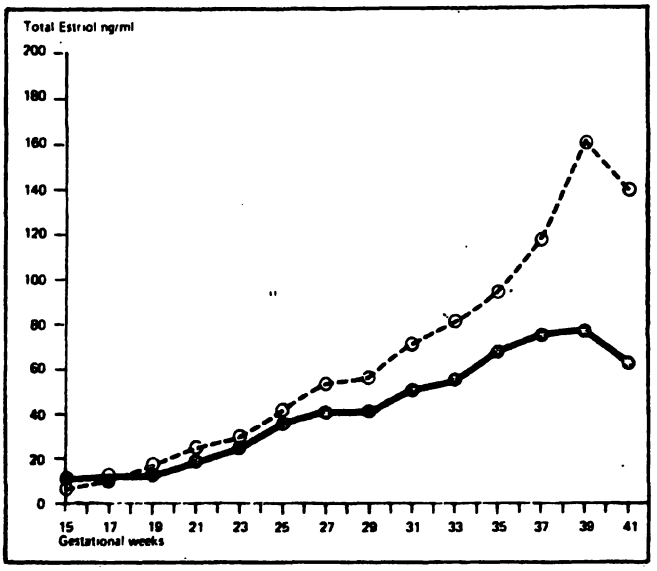

Fig. 3. Medians of total estriol levels in maternal plasma from 120 pregnancies with intrauterine growth retardation (lower solid line) and 463 pregnancies with normal outcome (upper broken line). Note the remarkably low total estriol increase in the group of intrauterine growth retardation following gestational week 30.

Tab. V. Means, medians, 95% and 70% confidence limits, and ranges of total estriol determined in 206 maternal plasma samples from 120 pregnancies with intrauterine growth retarded infants.

Gestational weeks	Number of plasmas assayed	Total estriol (ng/ml) Mean	Median	95% C.L.	70% C.L.	Min.-Max.
15–16	3	9.15	10.31	4– 13	5– 12	3– 14
17–18	5	12.39	12.23	5– 18	8– 16	4– 19
19–20	12	11.98	12.19	5– 19	9– 15	4– 17
21–22	13	19.03	17.99	7– 36	9– 29	6– 37
23–24	10	27.50	23.91	14– 59	15– 41	13– 64
25–26	19	43.82	35.66	18– 94	28– 68	12– 99
27–28	18	51.81	40.55	23– 99	29– 79	19–109
29–30	18	47.48	40.10	15–101	26– 72	12–106
31–32	4	49.67	49.98	22– 76	30– 69	20– 77
33–34	17	60.63	54.63	27–121	41– 80	25–129
35–36	26	78.04	68.37	30–157	39–115	25–159
37–38	30	85.13	75.36	26–177	52–128	23–247
39–40	23	95.38	77.96	35–209	52–159	22–243
41–42	8	77.58	61.93	51–131	56– 81	50–177

Tab. VII. Absolute and relative distribution of total and unconjugated plasma estriol levels from the pregnancies with intrauterine growth retardation within the estriol percentiles systems from the pregnancies with normal weight infants. In parentheses number of plasma samples examined.

	Total plasma estriol percentiles from pregnancies with normal outcome					Unconjugated plasma estriol percentiles from pregnancies with normal outcome				
	< 2.5	2.5–15	15–85	85–97.5	> 97.5	< 2.5	2.5–15	15–85	85–97.5	> 97.5
Gest. weeks 15–28 n = 80	11.25% (9)	17.50% (14)	57.50% (46)	8.75% (7)	5.00% (4)	17.50% (14)	28.75% (23)	42.50% (34)	11.25% (9)	0% (0)
Gest. weeks 29–42 n = 126	16.67% (21)	24.60% (31)	53.97% (68)	1.59% (2)	3.17% (4)	7.94% (10)	26.19% (33)	61.11% (77)	3.97% (5)	0.79% (1)
Gest. weeks 15–42 n = 206	14.56% (30)	21.84% (45)	55.34% (114)	4.37% (9)	3.89% (8)	11.65% (24)	27.18% (56)	53.88% (111)	6.80% (14)	0.49% (1)

2.4 Unconjugated estriol in pregnancies with intrauterine growth retardation

The concentrations of unconjugated estriol in maternal plasma from pregnancies with IUGR showed a rather similar trend towards their total estriol counterparts. They were substantially lower in almost all gestational weeks of the 2nd and 3rd trimester compared with their reference values of the normal outcome group (Tab. VI). The unconjugated estriol medians of the IUGR group lay, with the exception of one value in the early 2nd trimester, always below those of the normal outcome group. As early as the 2nd trimester the divergence of the two median curves was very clear, while in the 3rd trimester it did not appear to be as accentuated as it was in the case of total estriol (Fig. 4).

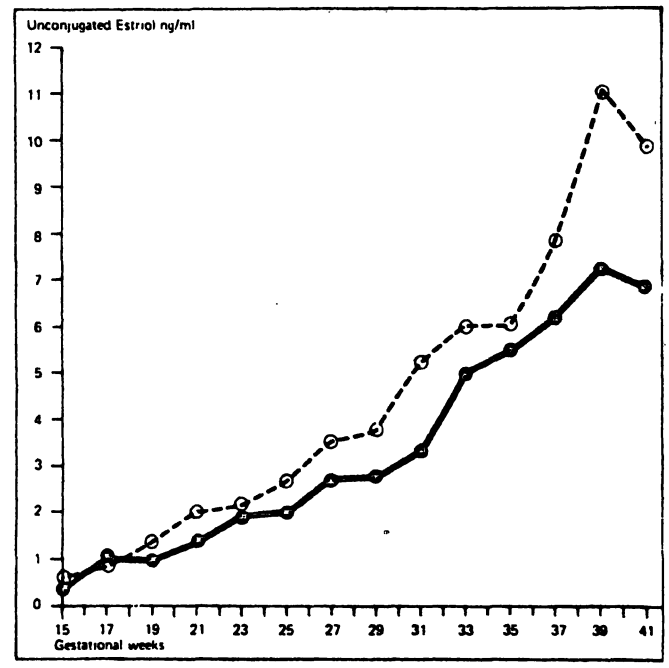

Fig. 4. Medians of unconjugated estriol levels in maternal plasma. Same patients as represented in Fig. 3. Lower solid line = IUGR pregnancies, upper broken line = pregnancies with normal outcome.

As can be seen from Tab. VI, VII, in the IUGR group 38.83% of unconjugated estriol values (11.65% plus 27.18%) could be detected below the 15th estriol reference percentiles (gestational weeks 15 to 42), while the corresponding value for total estriol was 36.4% (no statistically significant difference between the two values).

During the 2nd trimester the relative frequency of unconjugated estriol values in the IUGR group encountered below the 15th estriol reference percentile was 46.25% (17.50% plus 28.75%). This is in contrast to only 34.13% during the 3rd trimester (7.94% plus 26.19%); (Tab. VII).

2.5 Total and unconjugated estriol in pregnancies with intrauterine growth retardation

Fig. 5 demonstrates that in the course of the 2nd as well as of the 3rd trimester both total and unconjugated plasma estriol levels were significantly lower in the IUGR group compared with the estriol levels in the group with normal outcome. Separate evaluations of total and unconjugated estriol concentrations in women with severe or mild forms of fetal growth retardation (infants below the 3rd or the 10th birthweight percentiles) did not reveal any clear differences between the

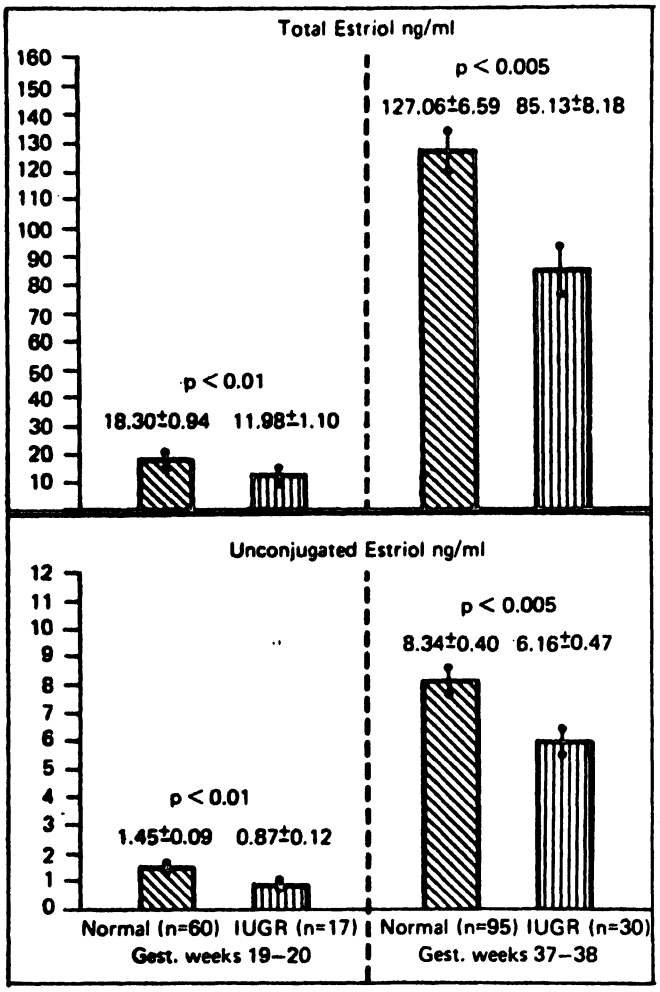

Fig. 5. Paired total estriol and unconjugated estriol levels (mean ± S.E.M.) in maternal plasma determined in the gestational weeks 19 to 20 and 37 to 38 in women with a normal or growth retarded fetus. Pregnancies with a growth retarded fetus have significantly lower total and unconjugated estriol levels (χ^2-test) both in the 2nd and in the 3rd trimester, compared with the estriol levels in women with a normal fetus.

Tab. VI. Means, medians, 95% and 70% confidence limits, and ranges of unconjugated estriol determined in 206 maternal plasma samples from 120 pregnancies with intrauterine growth retarded infants.

Gestational weeks	Number of plasmas assayed	Unconjugated estriol (ng/ml)				
		Mean	Median	95% C.L.	70% C.L.	Min.-Max.
15–16	3	0.40	0.33	0.1– 0.7	0.2– 0.6	0.09– 0.75
17–18	5	0.99	1.06	0.4– 1.5	0.6– 1.3	0.36– 1.50
19–20	12	0.87	0.96	0.3– 1.5	0.5– 1.3	0.28– 1.53
21–22	13	1.19	1.35	0.3– 2.2	0.5– 1.8	0.31– 2.36
23–24	10	2.09	1.91	0.9– 4.0	1.2– 3.2	0.89– 4.13
25–26	19	2.26	1.94	1.0– 4.8	1.3– 2.8	1.07– 5.25
27–28	18	2.88	2.71	0.9– 5.1	1.6– 4.2	0.82– 5.30
29–30	18	3.00	2.70	1.2– 7.1	1.8– 3.9	0.90– 8.51
31–32	4	3.27	3.26	2.7– 3.9	2.8– 3.8	2.64– 3.92
33–34	17	4.82	5.03	2.0– 7.6	3.0– 6.9	1.51– 7.61
35–36	26	5.76	5.45	1.9–11.8	3.1– 8.9	1.78–13.50
37–38	30	6.16	6.20	2.6–11.6	3.6– 8.0	2.45–14.67
39–40	23	7.24	7.20	2.5–13.7	4.0–10.0	2.16–14.09
41–42	8	7.25	6.86	4.7–10.4	5.6– 9.1	4.50–10.65

hormone levels in the two weight categories of IUGR pregnancies. Nor did the estriol levels from pregnancies with "pure" IUGR or IUGR associated with toxemia characteristically differ from each other.

The failure of serial low plasma estriol levels to show the characteristic rise which is commonly observed during the 3rd trimester of pregnancies with normal outcome was described by KLOPPER, JANDIAL and WILSON [21] as a reliable sign of intrauterine growth retardation. Fig. 6 shows that a similar plasma estriol conduct may be seen already during the 2nd trimester of pregnancies with IUGR.

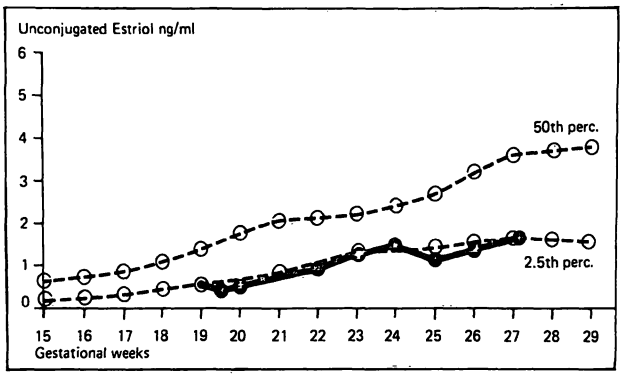

Fig. 6. Serial low unconjugated plasma estriol levels with reduced rising tendency (solid line) during the 2nd trimester in a 42 year-old women with a growth retarded fetus. At gestational week 38 delivery of a male infant of 2680 g in good health. – Broken lines = 2.5th and 50th unconjugated estriol percentiles in the group of pregnancies with normal outcome.

3 Discussion

The paired hormone assays demonstrated that both total estriol and unconjugated estriol concentrations in maternal plasma tended to be low in pregnancies with intrauterine growth retardation. Under the premise that the 15th percentiles of maternal plasma estriol concentrations in pregnancies with normal outcome were used as screening limits, 36.40% of the blood specimens from IUGR pregnancies had abnormally low concentrations of total estriol, and 38.83% of them had abnormally low concentrations of unconjugated estriol in the course of the 2nd and 3rd trimester. In the 2nd trimester the predictive value for IUGR of unconjugated plasma estriol was higher than that of total plasma estriol. In the 3rd trimester the situation was reversed. A clear superiority in predicting IUGR by unconjugated plasma estriol (in comparison with total plasma estriol), as has been claimed on theoretical considerations by some authors [30], could not be confirmed in this study.

The prediction of fetal growth retardation by plasma estriol estimations could be made with a similar degree of probability during the 2nd trimester as during the 3rd; an observation that may attract the attention of the obstetrician and perhaps of the perinatologic pathologist, since it sheds some light on the early onset of this gestational complication.

Studies of plasma estriol with the aim of predecting or monitoring intrauterine growth retardation have been conducted repeatedly [1, 3, 12, 15, 17, 18, 21, 22]. All these investigations, which dealt almost entirely with IUGR cases in late pregnancy, led to the unequivocal conclusion that in pregnancies with retarded fetal growth the maternal estriol values were commonly lower than normal and that the estriol curves obtained by serial plasma estriol measurements were flattened in comparison with those in pregnancies of comparable gestational age with normal outcome.

Less unanimity among the authors existed as to the rate of true IUGR prediction by plasma estriol. The quotation of accurate predictions by plasma estriol assays ranged from 20% reported by TOWLER et al. [28] to an astonishingly high 86% reported by GOEBEL et al. [13] in cases of IUGR without hypertension. Evidently some of the discrepancies in the assessment of the predictive value of plasma estriol measurements were due to application of different criteria in defining abnormally low estriol levels or birthweight limits for small-for-dates infants or the use of different hormone assay procedures.

Between the plasma estriol concentrations in pregnancies with normal outcome and those with IUGR there was a large area of overlapping, a fact which logically limits the diagnostic usefulness of the measurements. It presently often remains obscure why in one individual case of IUGR the classical low estriol values are found and in another case they are not. In this connection one should

keep in mind that the clinical syndrome of intrauterine growth retardation can result from diseases of the mother, the placenta and the fetus, or from special circumstances relating to genetics or maternal environmental setting.

Taking these various roots of IUGR into consideration, it should not be surprising that only a proportion of all IUGR cases can be discovered by feto-placental function tests such as maternal plasma estriol assay. For example, in a pregnancy complicated by ample placental infarcts and a substantial reduction in maternal-placental substrate and gas exchange, the investigator may easily find retarded fetal growth associated with abnormally low plasma estriol levels. On the other hand, a pregnant woman who is a severely undernourished drug user with a growth retarded fetus may have normal plasma estriol levels.

In the 2nd and 3rd trimester of gestation maternal plasma estriol is almost entirely a product of the conceptus. Being synthesized by complementary steroidogenic mechanisms in the fetus and placenta, estriol is released from the circulation of the conceptus into the circulation of the pregnant woman. Regular feto-placental synthesis of estriol requires a sufficient supply of oxygen and steroidal precursors; thus, plasma estriol assays may be used in the assessment of feto-placental function during the 2nd and 3rd trimester of pregnancy [6]. Normal maternl plasma estriol concentrations with a rising tendency in the progression of gestation indicate good outcome.

Since plasma estriol measurements can now be carried out readily, precisely, repeatedly and without major molestation to the mother, they are a valuable tool in monitoring and managing pregnancies with chronic complications such as intrauterine growth retardation, placental insufficiency or postmaturity. Assays of total estriol in amniotic fluid seem to be superior to maternal plasma estriol assays in predicting IUGR in late gestation [2]. Of course, the combined application of different clinical tests such as ultrasonography, fetal heart monitoring, HPL assays, total estriol, lecithin determinations in amniotic fluid and plasma estriol assays will provide the obstetrician engaged in the management of an IUGR pregnancy with much more useful data than would be obtained with an estriol determination alone.

Summary

In 913 plasma samples collected from 583 mothers in the 2nd and 3rd trimester of pregnancy (gestational weeks 15 to 42), paired determinations of total and unconjugated estriol levels were performed by radioimmunoassay. 707 of the samples were taken from 463 mothers who were delivered of healthy singleton normal birthweight infants. The remaining 206 plasmas assayed came from 120 pregnant women who gave birth to singleton small-for-date infants whose birthweight fell below the 10th percentile for gestational age and who were therefore classified as suffering from intrauterine growth retardation (IUGR).

From the levels encountered in the pregnancies with normal weight outcome, reference values such as medians, means, 95% and 70% confidence limits of total and unconjugated plasma estriol were calculated for the 2nd and 3rd trimester. Due to the asymmetrical distribution of the estriol data, their mathematical representation was predominantly done in percentiles. Values which fell below the 15th plasma estriol percentile for gestational age were regarded as abnormally low and indicators of potential fetal danger.

In pregnancies with IUGR, total and unconjugated estriol in maternal plasma tended to be lower than in pregnancies with normal weight offspring. The medians of total and unconjugated plasma estriol from the IUGR group lay usually below those from the normal birthweight reference group. During the third trimester the rate of abnormally low total estriol levels from the IUGR group amounted to 41%, while that of abnormally low unconjugated estriol levels was 34%. During the second trimester 29% of the total estriol values and 46% of the unconjugated estriol values from the IUGR group fell below the corresponding 15th estriol percentiles. Generally, the rate of abnormally low maternal plasma estriol levels from the IUGR group in the 2nd and 3rd trimester was almost identical – between 35% and 40% – for the total and the unconjugated estriol.

Three main conclusions could be drawn from this study: 1. Measurements of total and unconjugated estriol in maternal venous plasma during the 2nd and 3rd trimester of pregnancy were of comparable clinical usefulness. The overall efficiency of the two estriol tests in discriminating pregnancies with IUGR was 36% for total estriol and 38% for unconjugated estriol. 2. Predictions of IUGR by abnormally low plasma estriol levels could be made with a similar rate of accuracy both during the 2nd and 3rd trimester. 3. Plasma estriol determinations appeared to be most useful when normal estriol results provided some reassurance to the obstetrician that the current status of the fetus was satisfactory.

Keywords: Birthweight, intrauterine growth retardation, maternal plasma, monitoring of pregnancy, total estriol, unconjugated estriol.

Zusammenfassung

Paarige Bestimmungen des Gesamtöstriols und unkonjugierten Östriols im mütterlichen Plasma im 2. und 3. Trimenon – Ihre Beziehung zur intrauterinen Wachstumsretardierung

In 913 Plasmaproben von 583 Müttern im 2. und 3. Trimenon (Schwangerschaftswochen 15 bis 42) wurden paarige RIA-Bestimmungen von Gesamt- und unkonjugiertem Östriol vorgenommen. 707 Proben stammten von 463 Müttern, die von gesunden, normal wiegenden Kindern entbunden wurden. Die übrigen 206 untersuchten Plasmen waren von 120 Schwangeren, die small-for-date Kinder gebaren, deren Geburtsgewicht unterhalb der dem Schwangerschaftsalter entsprechenden 10. Perzentile lag und die deshalb als Kinder mit intrauteriner Wachstumsretardierung klassifiziert wurden.

Aus den Hormonwerten der Schwangerschaften mit normal wiegenden Kindern wurden Referenzwerte berechnet, d.h. Mediane, Mittelwerte, 95% und 70% Vertrauensbereiche von Gesamt- und unkonjugiertem Östriol für das 2. und 3. Trimenon. Wegen der asymmetrischen Verteilung der Werte erfolgte ihre mathematische Darstellung vorwiegend in Perzentilen. Werte, die sich unterhalb der dem Schwangerschaftsalter entsprechenden 15. Plasmaöstriol-Perzentile bewegten, wurden als abnorm niedrig angesehen und als Hinweiszeichen potentieller Gefährdung des Feten gewertet.

In Schwangerschaften mit intrauteriner Wachstumsretardierung tendierten Gesamtöstriol und unkonjugiertes Östriol im mütterlichen Plasma zu niedrigeren Werten als in Schwangerschaften mit normal wiegender Nachkommenschaft. Die Östriol-Mediane der Schwangerschaften mit intrauteriner Wachstumsretardierung lagen gewöhnlich unter den Östriol-Medianen der Schwangerschaften mit normalem Geburtsgewicht der Kinder. Während des 3. Trimenons betrug der Anteil der abnorm niedrigen Gesamtöstriol-Werte bei den Schwangerschaften mit intrauteriner Wachstumsretardierung 41%, während der Anteil der abnorm niedrigen Werte für unkonjugiertes Östriol 34% ausmachte. Während des 2. Trimenons lagen 29% der Gesamtöstriol-Werte und 46% der Werte für unkonjugiertes Östriol in der Gruppe der Schwangerschaften mit intrauteriner Wachstumsretardierung unter den entsprechenden 15. Östriol-Perzentilen der Referenzgruppe. Betrachtete man die Östriol-Werte aus dem 2. und 3. Trimenon gemeinsam, dann war der Anteil von abnorm niedrigen Östriol-Werten in der Gruppe der Schwangerschaften mit intrauteriner Wachstumsretardierung für Gesamtöstriol und unkonjugiertes Östriol nahezu identisch (zwischen 35% und 40%).

Drei hauptsächliche Schlußfolgerungen wurden aus der vorliegenden Studie gezogen: 1) Bestimmungen von Gesamtöstriol und unkonjugiertem Östriol im mütterlichen venösen Plasma waren während des 2. und 3. Trimenons von vergleichbarem klinischem Nutzen. Bei der Abgrenzung von Schwangerschaften mit intrauteriner Wachstumsretardierung betrug die Gesamttrefferquote der zwei Östrioltests 36% für Gesamtöstriol und 38% für unkonjugiertes Östriol im Plasma. 2) Vorhersagen von intrauteriner Wachstumsretardierung ließen sich aufgrund von abnorm niedrigen Östriolkonzentrationen im mütterlichen Plasma sowohl im 2. wie auch im 3. Trimenon mit ähnlicher Trefferquote machen. 3) Östriolbestimmungen im mütterlichen Plasma schienen sehr nützlich zu sein, wenn die normalen Östriol-Werte dem Geburtsmediziner einige Sicherheit vermittelten, daß der aktuelle Zustand des Fetens zufriedenstellend war.

Schlüsselwörter: Geburtsgewicht, Gesamtöstriol, intrauterine Wachstumsretardierung, mütterliches Plasma, Schwangerschaftsüberwachung, unkonjugiertes Östriol.

Résumé

Déterminations couplées de l'estriol plasmatique maternel total et non conjugué pendant le deuxième et le troisième trimestre de la grossesse – Relations avec le retard de croissance intra-utérin

Les dosages couplés de l'estriol total et de l'estriol non conjugué ont été réalisés par radio-immunologie sur 913 plasma de 583 mères au cours du 2ème et 3ème trimestre de la grossesse (15ème à 42ème semaines gestationnelles) – 707 échantillons plasmatiques ont été prélevés chez 463 mères ayant accouché d'enfants uniques de poids de naissance normal et en bonne santé. Les 206 restants ont été prélevés chez 120 femmes enceintes ayant accouché d'enfants uniques hypotrophiques avec un poids de naissance inférieur au dixième percentile pour l'âge gestationnel et ainsi considérés comme ayant subi un retard de croissance intra-utérin (RCIU).

A partir des résultats obtenus pour les grossesses ayant abouti à un nouveau-né de poids normal, on a calculé des valeurs de référence telles que les moyennes, la validité, les intervalles de confiance à 95% et 70%, pour les estriols totaux et les estriols non conjugués au second et au troisième trimestre. En raison de la distribution asymétrique des résultats, leur expression mathématique a été effectuée essentiellement en percentiles. Les valeurs situées sous le 15ème percentile de l'estriol plasmatique pour l'âge gestationnel ont été considérées comme anormalement basses et comme indicatrices de danger potentiel pour le foetus.

Lors des grossesses avec RCIU, l'estriol plasmatique maternel total et non conjugué tend à être plus bas que lors des grossesses ayant abouti à un enfant de poids normal. Les moyennes d'estriol plasmatique total et non conjugué dans le groupe avec RCIU sont en règle inférieures à celles du groupe de référence sans hypothrophie. Pendant le troisième trimestre le taux d'estriol total anormalement bas est de 41% dans le groupe avec RCIU, alors que le taux d'estriol total non conjugué est de 34%. Pendant le second trimestre, 29% des valeurs de l'estriol total et 46% des valeurs de l'estriol non conjugués sont situés sous le 15ème percentile dans le groupe avec RCIU. Globalement, la fréquence de taux d'estriol anormalement bas dans le groupe avec RCIU pendant le 2ème et le 3ème trimestre est à peut près la même – entre 35 et 40% – pour l'estriol plasmatique maternel total et pour l'estriol non conjugué.

On peut tirer trois conclusions principales de ce travail: 1. les dosages de l'estriol plasmatique maternel total et de l'estriol non conjugué revêtent une utilité clinique comparable pendant le 2ème et le 3ème trimestre. L'efficacité globale des deux dosages pour déterminer les grossesses avec RCIU est de 36% pour l'estriol total et de 38% pour l'estriol non conjugué. 2. La prévision du RCIU par la détermination de taux d'estriols plasmatiques anormalement bas peut être portée avec une fiabilité identique pendant le second et le troisième trimestre. 3. C'est lorsque les résultats sont normaux que les dosages de l'estriol plasmatique revêtent la plus grande utilité en assurant quelque peu aux obstétriciens que l'état actuel du foetus est satisfaisant.

Mots-clés: Estriol non conjugué, estriol total, hypotrophie, plasma maternel, poids de naissance, surveillance de la grossesse.

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Prof. Dr. med. G. Bacigalupo
Arbeitsgruppe Perinatale Medizin
Freie Universität Berlin
Mariendorfer Weg 28–38
D-1000 Berlin 44